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Recent progress on carotenoid metabolism in animals

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Abstract - The influence of dietary astaxanthin, canthaxanthin and zeaxanthin on the carotenoid content and composition of the oil droplets in chicken retina was investigated. From a "racemic" astaxanthin mixture, the (3S,3'S)-isomer was deposited almost selectively in the retina. Both oxidative and reductive metabolic pathways were followed by all three carotenoids. Astaxanthin, the main carotenoid in avian oil droplets, was obviously formed from both dietary zeaxanthin and canthaxanthin.
- Egg yolk pigmentation was studied in relation to carotenoid structure. Depositon rates and metabolites of some C_{30} and C_{40} carotenoids have been determined. Beta- and ψ-apo-carotenoids with a terminal methyl group in the γ-position were gradually shortened by a type of β-oxidation.
- Various yellow metabolites of astaxanthin have been identified in the prawn *Penaeus vannamei* and their absolute configurations determined. The 4,4'-oxo groups of astaxanthin were reduced stereospecifically, resulting in (4S,4'S)-tetrahydroxypirardixanthin. The presence of the novel, naturally occurring isoastaxanthin [(6S,6'S)-4,4'-dihydroxy-ε,ε-carotene-3,3'-dione] offered an explanation for a racemization of astaxanthin *in vivo*, which was proved in *Penaeus japonicus* after administration of optically active [^{3}H]-labelled astaxanthin.

INTRODUCTION

In the past three years since the last Symposium, numerous publications have appeared which document the importance of carotenoids in the industrial farming of animals. By far the greatest number deal with the biological effects of carotenoids in cattle, poultry and aquaculture and are aimed at improving production and quality of the product. A relatively small number of papers deal with metabolic transformations of carotenoids and with their function on a molecular basis. Since, in this meeting, some of the experts in this field reported on their own investigations or reviewed results obtained on animal carotenoid metabolism, I shall present in this article some studies carried out in our own laboratories. These concern:
- deposition of dietary carotenoids and their metabolites in the chicken retina,
- influence of structure on the rate of deposition of carotenoids and metabolites in egg yolk,
- metabolites of astaxanthin in the prawn *Penaeus*.

CAROTENOIDS IN CHICKEN RETINA

In Boston, three years ago, we discussed the occurrence of some tissue-specific carotenoids in the avian retina and their possible formation in the chicken embryo (ref. 1). Those studies had emanated from a co-operation with Dr. Brian Davies, University of Wales, Aberystwyth, UK. We had seen that distinct, stereospecific carotenoids were responsible for the colour of the red, yellow and greenish oil droplets located in the photoreceptor cells, the cones. Avian oil droplets were assumed to improve acuity in colour vision and were classified by Goldsmith *et al.* by microphotometric measurements and by analytical procedures (ref. 2).
In 1984, Bethan Davies showed that labelled zeaxanthin was mobilized from the egg yolk to the retina of the chick embryo (ref. 3).

Deposition and transformation of dietary carotenoids in the chicken retina

Dr. Harald Weiser of the Roche Biochemical Animal Section carried out growth tests by feeding (3R,3'R)-zeaxanthin, "racemic" astaxanthin (i.e. a mixture of the stereoisomers RR:RS:SS = 1:2:1) and canthaxanthin to one-day-old chicks (strain Lohmann) (ref. 4). The control diet contained a minimal amount of 450 IU vitamin A and was virtually free of carotenoids. The experimental groups were fed astaxanthin, canthaxanthin or zeaxanthin in dosages of 36-144 mg/kg feed.

For supporting growth, canthaxanthin and zeaxanthin could replace vitamin A partly or entirely. Astaxanthin was no growth factor and the animals that were fed astaxanthin without vitamin A supplementation became weak and moribund and were sacrificed after 21 days. The chicks of the canthaxanthin and zeaxanthin groups were sacrificed after 39 days, the retinas removed and the retinal carotenoids analysed.

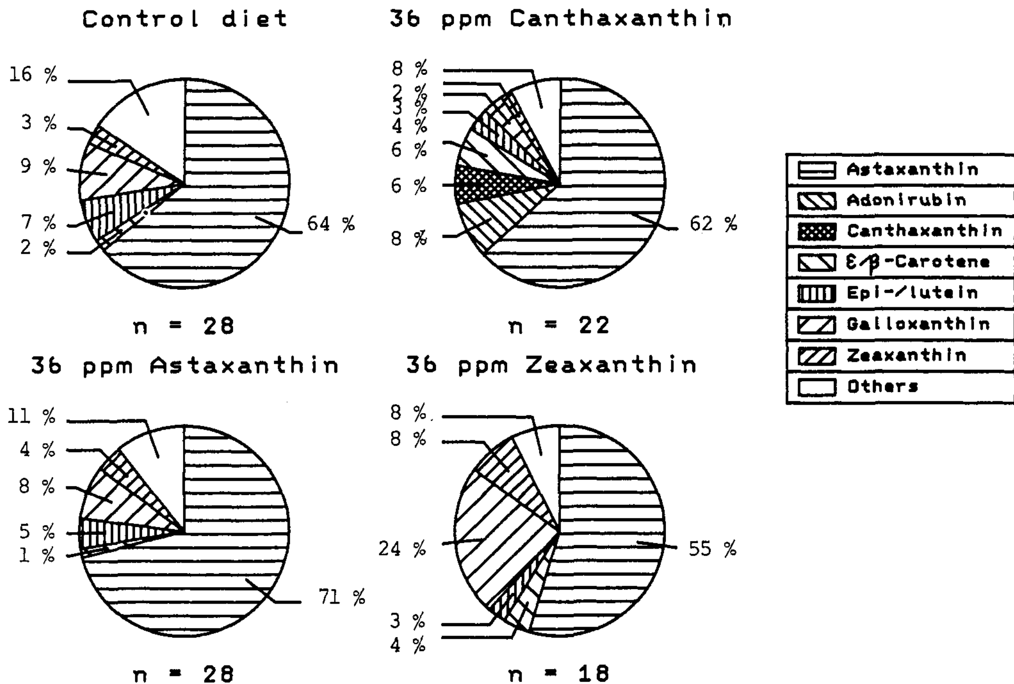

Fig. 1 Carotenoid composition in the retina of chickens fed canthaxanthin, astaxanthin, zeaxanthin or control diet.

Figure 1 presents the relative abundance of carotenoids in the retinas of the four groups that were fed 36 mg carotenoid/kg feed. It is evident that dietary astaxanthin and zeaxanthin did not alter the range of carotenoids present but only changed the ratio of the basic retinal carotenoids, by increasing the amounts of astaxanthin, galloxanthin, zeaxanthin, lutein of various chiralities and of ε,ε-carotene. In the canthaxanthin-fed group, not only were small amounts of this dietary carotenoid found, but also adonirubin and β,β-carotene, which are not usually encountered in the chicken retina (Figs. 2 and 3).

Based on quantitative analyses of the main retinal carotenoids, namely astaxanthin, zeaxanthin and galloxanthin including stereoisomers, the following metabolic processes may be assumed: all three dietary carotenoids were deposited in the retina; astaxanthin was partly metabolized to zeaxanthin and galloxanthin, zeaxanthin increased the amount of astaxanthin and galloxanthin. After administration of astaxanthin or zeaxanthin, a slight increase of ε,ε-carotene was also observed. Canthaxanthin obviously follows an oxidative pathway to astaxanthin as well as a reductive one to β,β-carotene. This is interesting from an evolutionary point of view; apparently, the capability of some crustaceans to transform canthaxanthin into astaxanthin (ref. 5) is preserved in the chicken retina, though this modification is lost in many fishes such as salmonids (ref. 6).

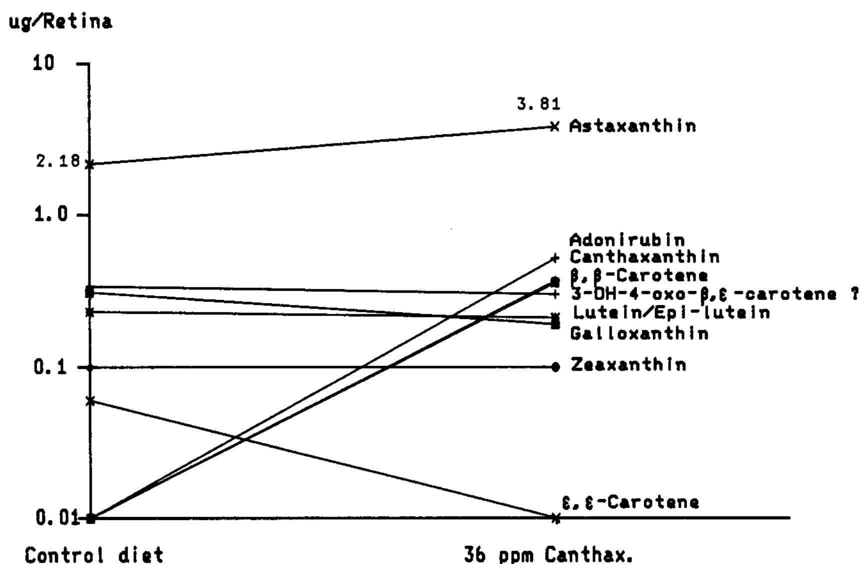

Fig. 2 Carotenoids in chicken retina (μg/retina) after canthaxanthin feeding (36 mg/kg feed) for 39 days

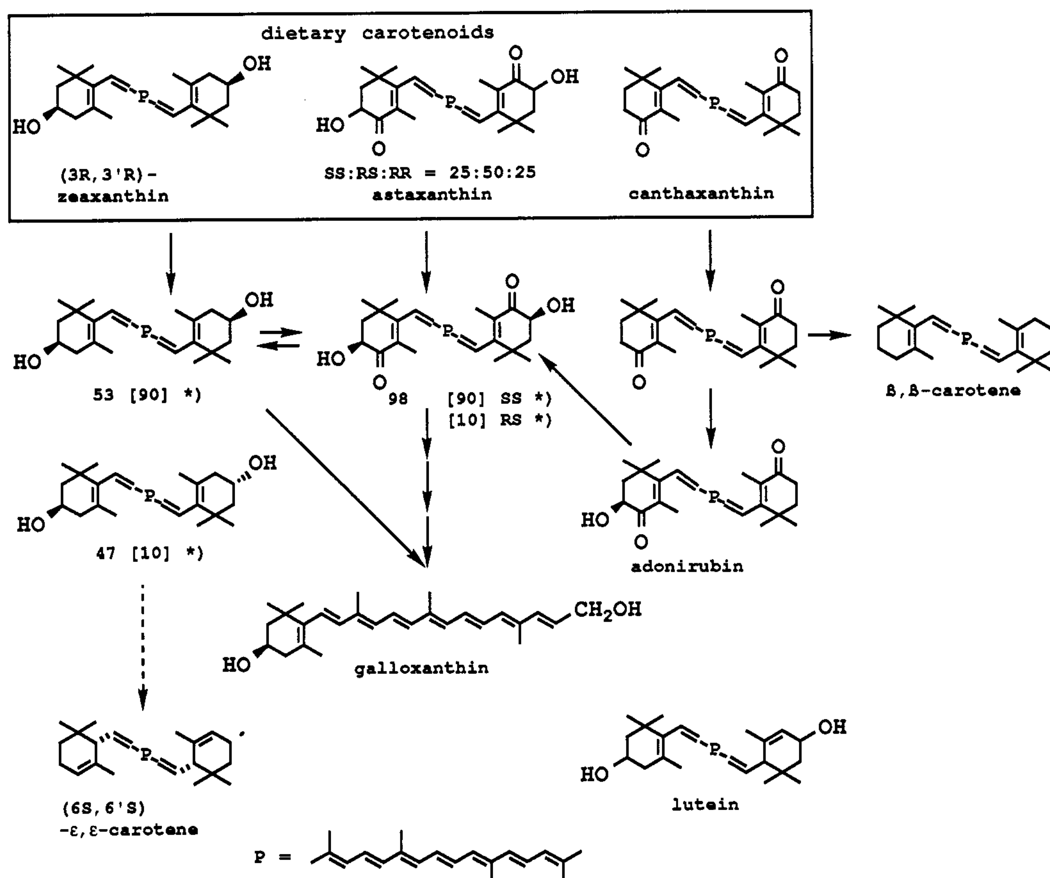

Fig. 3 Deposition and transformation of dietary carotenoids in chicken retina

*) without brackets: Percent of optical isomers in control animals
in brackets [ ]: Percent of optical isomers after carotenoid feeding

Selective absorption of (3S,3'S)-astaxanthin from a racemic mixture and non-racemization of (3R,3'R)-zeaxanthin

These studies may be open to criticism because they were carried out without radio-labelled compounds. However, by means of sophisticated analytical methods, and by isolation of the respective all-trans-isomers and subsequent derivatization (refs. 8, 9, 10) it was possible to determine the quantitative stereoisomeric composition. Table 1 clearly demonstrates that (3S,3'S)-astaxanthin was deposited preferentially, followed by (3R,3'S;meso)-astaxanthin. The (3R,3'R)-isomer from the dietary mixture was not deposited at all. Regarding zeaxanthin, we had, at the last Symposium, discussed the unexpected finding that zeaxanthin in chicken retina was a mixture of the (R,R)- and the meso-isomers. This was intriguing, as zeaxanthin had been considered a pre-cursor of astaxanthin which is optically pure (3S,3'S) (refs. 1, 10). From our experiments, it is now evident that the ingested (3R,3'R)-zeaxanthin was deposited as such; no racemization could be observed. It may therefore be concluded that the meso-isomer is a secondary metabolite of (3R,3'R)-zeaxanthin formed by a redox-system perhaps via 3'-dehydrolutein and lutein (ref. 1, 11). To determine why such an obviously species-specific equilibrium of xanthophyll stereoisomers is maintained in the eye and to establish the possible function of this equilibrium in vision requires further research.

Table 1 Quantity and configuration of astaxanthin and zeaxanthin in the chicken retina after administration of the respective carotenoid

Experimental groups	Astaxanthin in Retina						
	ng/retina	RR ng	(%)	RS ng	(%)	SS ng	(%)
Rac. Astax. *)	6250	0		625	(10)	5625	(90)
Control	4640	0		93	(2)	4547	(98)
Increase in astaxanthin	1610	0		532	(33)	1078	(67)
(3R,3'R)-Zeax. *)	3600	0		72	(2)	3528	(98)
Control	2340	0		42	(2)	2293	(98)
Increase in astaxanthin	1260	0		30	(2)	1235	(98)
	Zeaxanthin in Retina						
	ng/retina	SS ng	(%)	RS ng	(%)	RR ng	(%)
(3R,3'R)-Zeax. *)	500	0		50	(10)	450	(90)
Control	100	0		47	(47)	53	(53)
Increase in zeaxanthin	400	0		3	(1)	397	(99)

*) 36 mg/kg feed

CAROTENOIDS AS POTENTIAL EGG YOLK PIGMENTERS

The consumer expects high quality eggs not only direct from the farm but also in industrial poultry products. One mark of perceived egg quality is the yolk colour which depends almost entirely on the carotenoid content and composition in the layers' feed. The appealing appearance of an egg yolk depends not only on the colour hue but also on its saturation and the dominant wavelength that are also responsible for its luminosity.

Influence of carotenoid structure on absorption and deposition

The deposition rate in egg yolk has been tested for some acyclic, cyclic and apo-carotenoids of various structures synthesized by Drs. K. Bernhard and U. Hengartner (ref. 12).

Rather than merely a comparison of pigmenting efficacies by statistical means, some biochemical, analytical studies regarding deposition rate and metabolism have been undertaken. Deposition rate is defined as the amount deposited in egg yolk as a percentage of the quantity ingested. When a compound was converted into metabolites, the amounts of the original compound plus metabolites were summed and considered as total deposition. The aim was to obtain some basic knowledge about how structure influences absorption, deposition and metabolism.
The following compounds were added as beadlets to the basic feed (10 mg/kg) of laying hens (Shaver Starcross 288):
torularhodin ethyl ester 1, torularhodin 2, torularhodin aldehyde 3, β-apo-8'-carotenoic acid ethyl ester 4, β-apo-8'-carotenal 5, β-apo-2'-carotenal 7, 6'-apo-lycopenoic acid ethyl ester 16, 6'-apo-lycopenal 17, and 8'-apo-lycopenal 18. The pigmentation trial was carried out by Dr. J. Broz of our 'Animal Nutrition' department. Eggs were collected during days 16-20, when the yolk colour had reached plateau values; 20-30 yolks were pooled per group and the administered compound as well as the metabolites were analysed. Numerous chromatographic separations (column, TLC, HPLC) on adsorption and reversed phases were involved as well as chemical reactions and derivatizations such as esterification of the carboxylic acids with diazomethane and finally characterization by MS (Mr. W. Meister) and ^{1}H-NMR (Dr. G. Englert).

The results obtained from a first group, fed C_{40} and C_{30} carotenoids as pigmenting agents are compiled in Fig. 4.

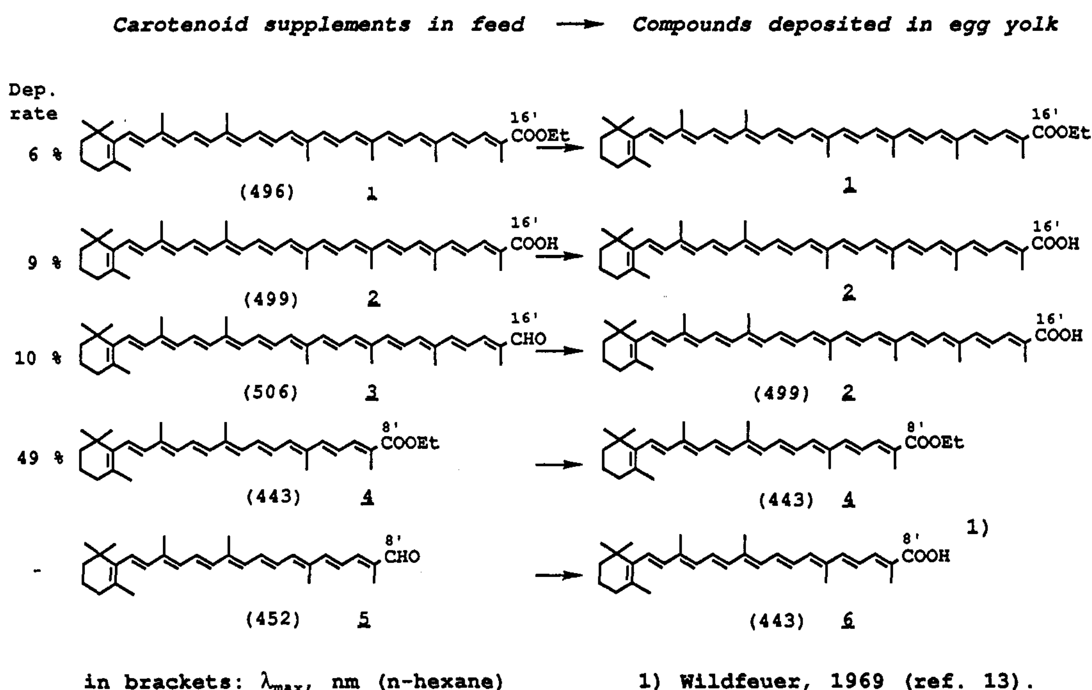

Fig. 4 C_{40} and C_{30} carotenoids 1-5 fed to laying hens and compounds deposited in egg yolk

It is known that the C_{40} hydrocarbons β,β-carotene and lycopene are virtually not deposited at all in egg yolk. By the introduction of oxygen functions into the molecule, however, the absorption rate can be improved markedly.

- From the comparison of the red C_{40} carotenoid 1 and the yellow C_{30} apo-carotenoid 4 (Fig. 4) it is evident that the length of the molecule significantly influences the deposition rate in egg yolk. However, the type of the functional group, aldehyde, carboxylic acid or ester, does not change the order of magnitude of the deposition rate.
- The ethyl esters 1 and 4 were deposited as such. Only minor amounts of 3-6% of the total deposited carotenoid had been hydrolyzed to the corresponding acid. The carboxylic acid 2 was deposited unchanged. The aldehydes 3 and 5, however, were oxidized to the respective carboxylic acids.

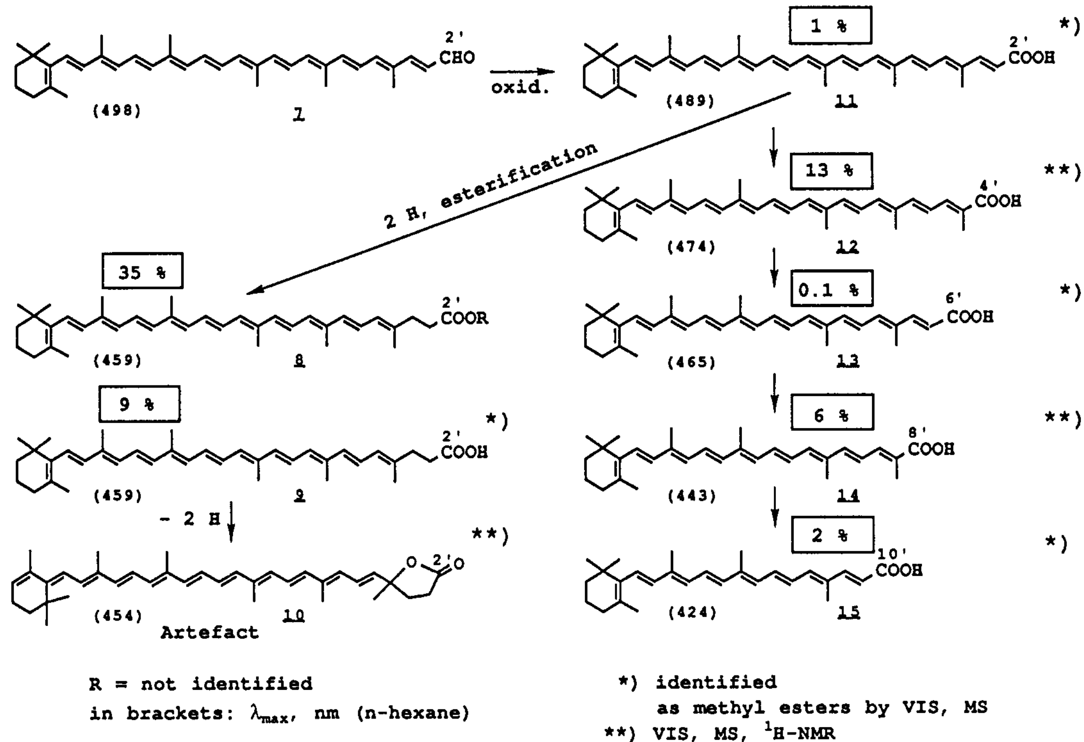

Fig. 5 β-Apo-2'-carotenal 7 (C_{37}) fed to hens and metabolites detected in egg yolk
Deposition rate in total: 9%,
in boxes: relative % of metabolites (not identified: 33 %)

- After feeding the C_{37} aldehyde 7 (Fig. 5), again none of the original material was found in egg yolk but only carboxylic acids. It was evident that these acids were by no means homogeneous. Another, comparatively apolar, fraction was found which at first was assumed to be a reduction product, namely a β-apo-carotenol with an undecaene structure according to its chromophore, but this fraction was found to consist of two compounds, an ester and a γ-lactone. Obviously, the latter must be considered as an artefact arising by spontaneous lactone ring closure of the carboxylic group with the γ-C of the 3',4'-dihydrogenated acid and concomitant retro-rearrangement of the polyene system. The question of why the chicken stabilizes the majority of this labile compound by esterification deserves further consideration. In contrast, xanthophyll or retinyl esters are hydrolyzed and deposited in egg yolk in their free form. Details of isolation and identification will not be presented but it should be mentioned that the carboxylic acids were esterified with diazomethane, the methyl esters separated by column chromatography on MgO according to the number of double bonds in the polyene system, the all-trans isomers of the single esters isolated by HPLC and finally characterized by MS and in some cases also by ^{1}H-NMR.

It should be noted that, after administration of the originally carmine red 2'-apo-carotenal, a number of carotenoids with a much shorter conjugated polyene system ranging in colour from orange to bright yellow were found in egg yolk. As seen in Fig. 4, the C_{40} and C_{30} carotenoids 1, 2, 3, 4, 5 were not degraded in chain length in egg yolk. What is the structural difference between β-apo-2'-carotenal 7 and the homologues torularhodin aldehyde 3 and β-apo-8'-carotenal 5 that they should be metabolized so differently? Certainly, it seems not to be the length of the molecule but the fact that compound 7 possesses a γ-methyl group, whereas the others have an α-methyl, which obviously causes a steric hindrance to enzymic attack.

For further comparison, three acyclic model compounds 16, 17, 18 and their metabolites are depicted in Fig. 6. The relative proportions of the metabolites are marked in the corresponding boxes. Characterization was again carried out by VIS, MS and ^{1}H-NMR.

- Again, the apo-lycopenals 17 and 18 were oxidized to the corresponding carboxylic acids, but while 8'-apo-lycopenal 18 with its α-methyl substituent maintained its chain length, compound 17 with the methyl group in the γ-position was readily degraded to acids of shorter chain length.

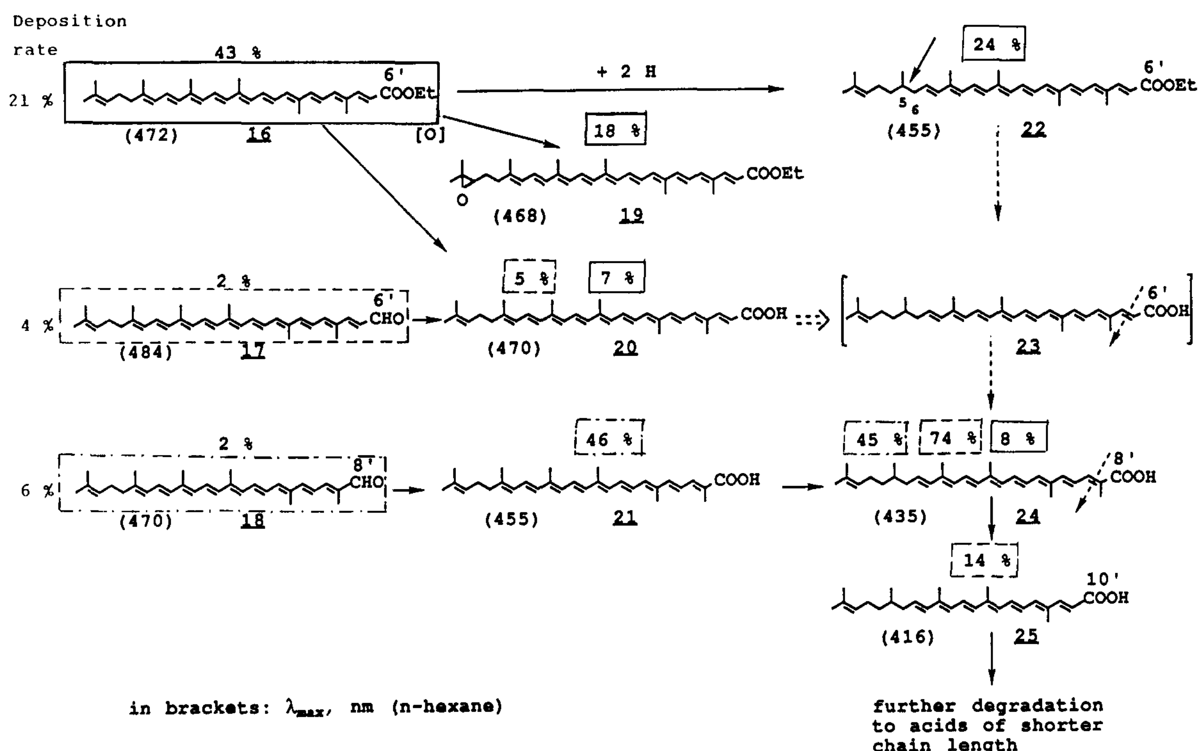

Fig. 6 6'-Apo-lycopenoic acid ethyl ester 16, 6'-apo-lycopenal 17 and 8'-apo-lycopenal 18 fed to laying hens and identification of metabolites in egg yolk

- Another metabolic peculiarity of these acyclic carotenoids was hydrogenation in the 5,6-position.
- The 6'-apo-lycopenoic acid ethyl ester 16 showed a much higher deposition rate (21 %) than the apo-lycopenals 17 and 18 (4% and 6%). In this case, however, the ethyl ester 16 was only partly deposited unchanged. In part, epoxidation occurred in the 1,2-position, partly the ester was hydrolyzed and hydrogenated in the 5,6-position. Finally, once hydrolyzed, the 5,6-dihydrogenated 6'-carboxylic acid 23 was also shortened by two carbons resulting in 5,6-dihydro-8'-apo-lycopenoic acid 24.

Comparative metabolic considerations

It has been shown that some carotenoids tested as egg yolk pigmenters were metabolized, while others were not.

- The ethyl esters 1 and 4 of the C_{40} carotenoid torularhodin and of the C_{30} β-apo-carotenoic acid were both deposited unchanged in egg yolk. 6'-Apo-lycopenoic acid ethyl ester 16 with the methyl group in the γ-position was hydrolyzed and subsequently underwent shortening by β-oxidation. Obviously, the hydrolase can attack carotenoids without a terminal methyl group more readily.
- The apo-carotenals were oxidized and deposited as carboxylic acids; again, those carboxylic acids 2, 6, 21 that arose from an aldehyde with a terminal methyl group were not degraded further, whereas those originating from aldehydes 7, 17 with a methyl group in the γ-position were gradually oxidized by two and three carbons, respectively.
- Hydrogenation of the 5,6-double bond occurred in the acyclic apo-carotenoids 16, 17, and 18. β-Apo-2'-carotenoic acid 11 was hydrogenated in the 3',4'-position. The loss of up to four double bonds gives a chromophore which exhibits quite different pigmenting characteristics.

An explanation for this structure-specific behaviour may be offered by comparing the enzymes involved in the β-oxidation of fatty acids (ref. 14). When the enzymic cascade of fatty acid oxidation is compared with that of carotenoic acids, two things become apparent; First, the oxidation itself is an intramitochondrial process and, secondly, for the passage of the fatty acyl moiety through the mitochondrial membrane, two enzymic steps are required, namely activation to acyl-CoA and then transfer as a carnityl-acyl-complex.
In the case of carotenoic acids, these may be the limiting steps. Thus if activation to the acyl-CoA is not possible because of steric hindrance by the terminal methyl group, then passage through the inner mitochondrial membrane would not be possible. In turn, if carotenoic acids with their methyl group in the γ-position can form the activated acyl-CoA, then the acyl group may enter the mitochondria by means of the carnitine-acyl transferase, and only then would the cascade of chain-shortening be possible.
In our studies on egg yolk pigmentation, neither the sites of these conversions, presumably intestinal mucosa or liver, nor the question of whether further degradation to retinoic acid occurs, were investigated. Retinal and retinol were not measured. However, these studies may be an example of an eccentric cleavage of β-apo-carotenoids in non-mammals. It may be asked whether the chain shortening as shown above is due to an unspecific dioxygenase or to enzymes that are also involved in the β-oxidation of fatty acids. Possibly, glucuronides are formed and then excreted. The presence of the C_{37} γ-lactone 10, originally formed from the 3',4'-dihydrogenated acid 9, may show some analogy to vitamin A metabolism where similar shorter lactone metabolites have been found in bile and urine of rats and humans (ref. 15).

METABOLITES OF ASTAXANTHIN IN THE CRUSTACEAN PENAEUS

Tetrahydroxypirardixanthin (isolated from *P. vannamei*) (5,6,5,'6'-tetrahydro-β, β-carotene-3,4,3',4'-tetrol)

At the previous Symposium, we reported the occurrence of yellow carotenoid esters (20-30%) besides the main astaxanthin esters in some wild species of Penaeidae (ref. 1). Those yellow carotenoids were not identical with lutein or zeaxanthin, but a major part corresponded to 3,4,3',4'-tetrols with 5,6-dihydro-β-rings. The same 5,6-saturated tetrols have previously been reported by Matsuno et al. in the spindle shell Fusinus perplexus (ref. 16), in three species of Fusinus and also in the prawn Penaeus japonicus by Katagiri et al. (refs. 17, 18). In none of those papers was the absolute configuration described. We considered the assignment of the absolute configuration of these tetrols with 8 centres of asymmetry something of a challenge, particularly that for the one isolated from Penaeus, where the compound is assumed to be the reduction product of astaxanthin. Since astaxanthin in these higher crustaceans of the order Decapoda is an almost "racemic" mixture of all three configurational isomers, different stereoisomers of tetrahydroxypirardixanthin could also be expected. We re-isolated the yellow fraction from the carapace of cultured Penaeus vannamei that had been fed racemic astaxanthin and compared the spectroscopic data (^{1}H-NMR, CD) with those of samples obtained from the wild species. The esters were again saponified and, for reasons of higher stability of the product, acetylated in the presence of the catalyst 4-dimethyl-aminopyridine (DMAP) (ref. 19). Dr. G. Englert assigned the relative configurations of these tetraacetates by ^{1}H-NMR spectroscopy and Dr. K. Noack determined CD spectra with minute amounts of the isolated material. However, only after the total synthesis of the optically active (3R,4S,5R,6R,3'R,4'S,5'R,6'R)-tetraacetoxypirardixanthin by our colleague Dr. U. Hengartner (ref. 20), could the spectra be assigned unequivocally. The metabolites depicted in Fig. 7 were of various absolute configurations and occurred in various combinations. All were characterized by MS, ^{1}H-NMR and CD.

The 4-hydroxy group exhibits the same configuration in both types of end-group, in the saturated cyclohexane rings A (3R,4S,5R,6R) and B (3S,4S,5R,6R) of tetrahydroxypirardixanthins 25, 26 and in the β-end-groups D (3R,4S) and E (3S,4S) of crustaxanthins 32 and 33. Obviously, the reduction of astaxanthin was again stereospecific, but opposite to that found in Atlantic salmon (refs. 1, 21).

In Boston, we reported the occurrence of the novel natural 4-hydroxy-ε-caroten-3-one end-group C (ref. 1), but only recently succeeded in isolating also the symmetrical isoastaxanthin 28. In 1981, a possible racemization of astaxanthin in vivo was suggested by Buchecker (ref. 22) and Zell et al. proposed a similar reaction mechanism for the synthesis of isoastaxanthin via the enediol (ref. 23). The biological product isolated from P. vannamei was optically active and exhibited the (6S,6'S)-configuration according to CD. Also the pirardixanthin derivatives showed uniformly the same configuration at C-6,C-6'.

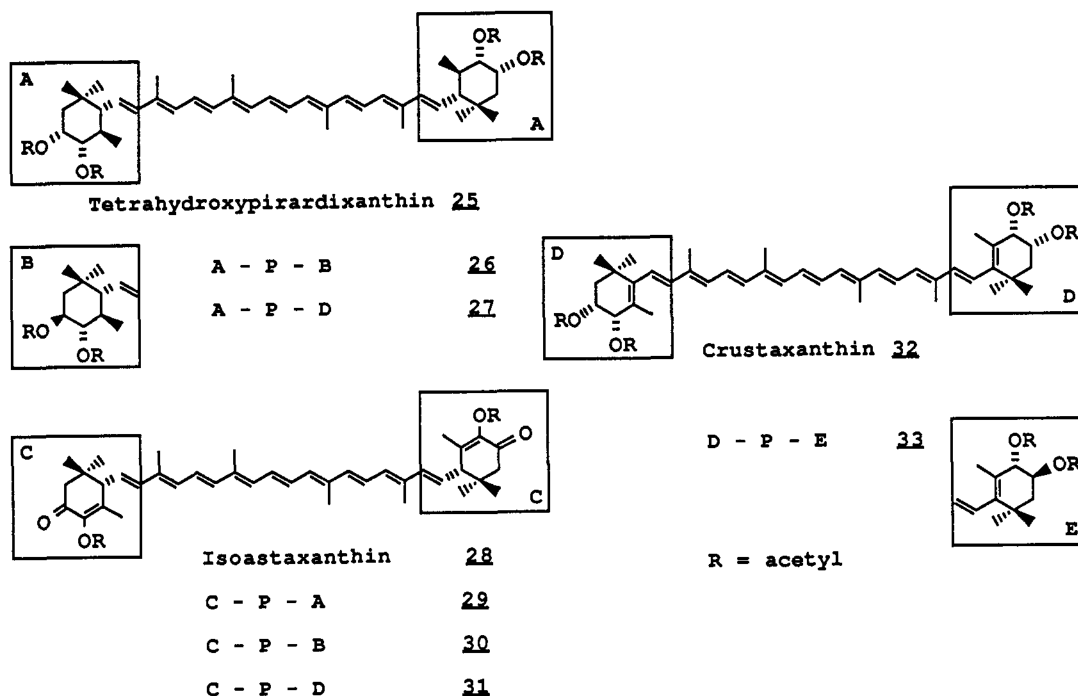

Fig. 7 Metabolites of astaxanthin in *Penaeus vannamei*: tetrahydroxypirardixanthin, isoastaxanthin, crustaxanthin and related compounds

Since in these higher crustaceans all three astaxanthin isomers were found, it was a challenge to investigate a possible racemization *in vivo*. Unfortunately, the number of animals was very small but, for the first time, it became possible to maintain the species of *P. japonicus* in our Basel laboratories and finally, the feeding trial was carried out successfully by Mr. P. Horne with some animals that survived quite well.

Experimental conditions and analytical procedures

$[15,15'\text{-}^3H_2]$-(3S,3'S)-Astaxanthin with a specific radioactivity of 198 µCi/mg or 439 600 dpm/µg dissolved in cod liver oil was applied onto pellets of a commercial feed (Gold coin). The astaxanthin supplement in the feed amounted to 200 ppm. The average live weight of the prawns (N = 5) was 10 grams at the beginning and 12.3 grams at the end of a 21-day feeding period.

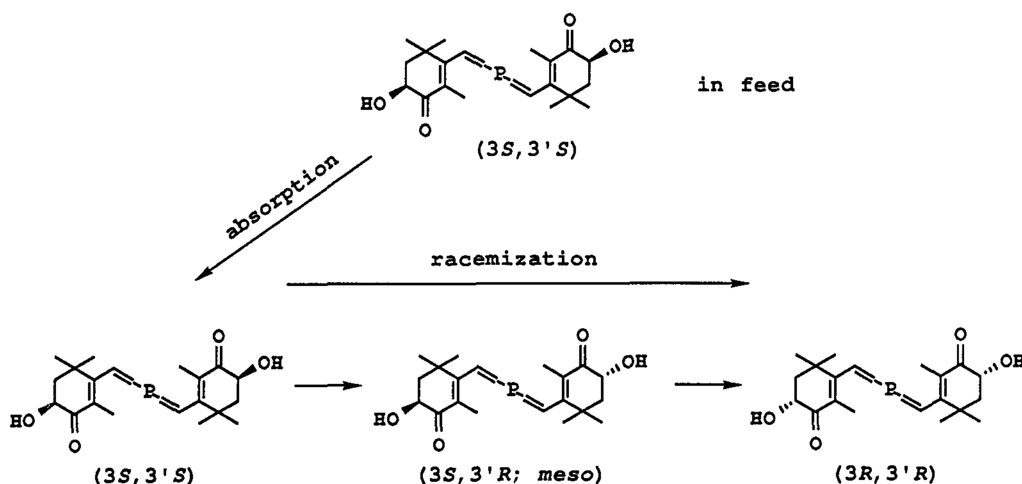

Fig. 8 *In vivo* racemization of optically active $[15,15'\text{-}^3H_2]$-(3S,3'S)-astaxanthin in *Penaeus japonicus*

Table 2 Isolation and analysis of astaxanthin, and mono- and diacetylenic asterinic acid from the carapace of *Penaeus japonicus*

SEPARATION AND ANALYSIS

1. Chrom. on SiO_2		μg (VIS)	dpm.10^6	spec. act. dpm/μg
Esters (carot. equiv.)		1 343	18.83	14 020
Free "astax."		223	8.18	36 680
total		1 566	27	
2. Prep. TLC on SiO_2				
"Astaxanthin"		160	7.29	45 560
Other carotenoids		39	0.22	5 640
3. HPLC, isolation of		**all-trans isomers**		
	area %	μg (VIS)	dpm.10^6	dpm/μg
7,8,7',8'-tetra-dehydro-astax.	28	20	0.06	2 140
7,8-didehydro-astaxanthin	19	15	0.11	5 790
Astaxanthin	53	43	4.06	94 420
4. HPLC of dicamphanates				
Astaxanthin			dpm	
(3*R*,3'*R*)	13	0.28	21 000	75 000
(3*R*,3'*S*;*meso*)	43	1.10	97 400	88 000
(3*S*,3'*S*)	44	1.39	157 000	113 000
7,8,7',8'-tetra-dehydro-astax.				
(3*R*,3'*R*)	25	2	1 000	500
(3*R*,3'*S*;*meso*)	46	4.3	200	50
(3*S*,3'*S*)	29	2.6	120	50
7,8-didehydro-astaxanthin				
(3*R*,3'*R*)	15	0.64	940	1 470
(3*R*,3'*S*;*meso*)	37	1.65	1 680	1 020
(3*S*,3'*S*)	48	2.1	2 480	1 180

Carotenoids were extracted with acetone from the carapaces, exclusively. The four steps of separation, isolation and analysis as well as the distribution of the radioactivity in the various fractions are summarized in Table 2. Chiral analysis of astaxanthin and asterinic acids was performed via the dicamphanate derivatives (ref. 8). It has only been possible so far to investigate in detail the free astaxanthin fraction, although a much larger portion of radioactivity was found in the esters. The free astaxanthin fraction showed a considerable specific radioactivity, which increased continuously during the purification procedure. Finally, a specific radioactivity of approximately 100 000 dpm/µg astaxanthin was attained, corresponding to a dilution of 1:4 with endogenous astaxanthin (Table 2, section 4).

The following conclusions may be drawn:
- The ratio of astaxanthin stereoisomers found was similar to that reported previously in P. vannamei (ref. 1) and in P. japonicus (ref. 18).
- The fact that an almost equal specific radioactivity was observed in all three stereoisomers, although only the (S,S)-isomer had been fed, allows the conclusion that racemization of the astaxanthin end-group took place during or after absorption.
- Moreover, it may be noted that a considerable portion of 7,8,7',8'-tetradehydro- and 7,8-didehydro-astaxanthin (28% and 19%) was present in the "astaxanthin fraction". From the fact that these acetylenic compounds are also "racemic" mixtures, two conclusions may be drawn: acetylenic compounds have not previously been considered as metabolites of astaxanthin, but are assumed to be transformation products of the algal carotenoids diatoxanthin [(3R,3'R)-7,8-didehydro-β,β-carotene-3,3'-diol] and alloxanthin [(3R,3'R)-7,8,7',8'-tetradehydro-β,β-carotene-3,3'-diol] in many aquatic animals. According to the very low specific activity found in the two acetylenic compounds in these prawns, also here 7,8-didehydro- and 7,8,7',8'-tetradehydro-astaxanthin may be excluded as metabolites of astaxanthin. However, it is surprising that the astaxanthin end-groups in these two compounds are no longer optically pure, but exhibit the same "racemic" mixture as seen in astaxanthin. This fact again supports the finding of a racemization of the astaxanthin end-group in vivo, in contrast to the optically pure asterinic acid found in members of the Euphausiaceae together with all three stereoisomers of astaxanthin (ref. 24).

We plan to work up also the labelled ester fractions (Table 2, section 1) that contain also the the yellow metabolites depicted in Fig. 7. It may then be possible to unravel metabolic pathways of astaxanthin in P. japonicus and to find out whether the yellow compounds are involved in the racemization of astaxanthin. If so, it would be another objective of research to find out whether those reactions occur at the level of free or esterified carotenoids or of carotenoproteins. - With these prospects, the specialists in the cultivation of crustaceans should be encouraged to pursue further the biochemistry of these fascinating animals.

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